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DATA EVALUATION RECORD

P-MENTHANE-3,8-DIOL

STUDY TYPE: POSTNATAL DEVELOPMENTAL NEUROTOXICITY STUDY - RAT [NON-GUIDELINE]

MRID 46342801

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

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Task No. 04-78

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DATA EVALUATION RECORD

EPA Secondary Reviewer: Roger Gardner 2/4/05

STUDY TYPE:	Postnatal Developmental Neurotoxicity - Rats (non-guideline)
MRID NO:	46342801
DP BARCODE NO:	DP 308571/4822526 and DP 308563/4822-515
CASE NO:	Not reported
DECISION NO:	
TEST MATERIAL:	p-Menthane-3,8-diol technical (EPA Reg No. 4822-526/ 4822-515; 99.63% a.i.)
PROJECT NO:	610-001
SPONSOR:	SC Johnson & Sons, Inc., 1525 Howe Street, Racine, WI
TESTING FACILITY:	Argus Research, 905 Sheehy Drive, Bldg. A, Horsham, PA
TITLE OF REPORT:	Percutaneous postnatal developmental neurotoxicity study of p-menthane 3, 8-diol in Crl: CD®(SD)IGS BR VAF/Plus® rats
AUTHOR:	J.F. Barnett
STUDY COMPLETED:	June 15, 2004
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	No evidence of developmental neurotoxicity was observed under the conditions of the study.
CLASSIFICATION:	ACCEPTABLE/NON-GUIDELINE

EXECUTIVE SUMMARY: In a postnatal developmental neurotoxicity study (MRID 46342801, P-menthane-3,8-diol (99.63% a.i., Lot # 061402Ad; Batch # 463D3) was administered percutaneously to 80 Crl:CD®(SD)IGS BR VAF/Plus® rats/sex/group from postnatal day (PND) 10 through PND 21 at a dose of 0, 400, 800, or 1000 mg/kg/day. Dams were not treated. A Functional Operational Battery (FOB) was performed on 20 offspring/dose on PNDs 12, 22, 36, 46, and 61. Motor activity, auditory startle reflex habituation, and learning and memory testing (passive avoidance and watermaze performance) were evaluated in 20 rats/sex/group. Pup physical development was assessed by body weight, body weight gain, and food consumption (post-dosing). The age of sexual maturation (vaginal opening in females and preputial separation in males) was recorded. Sacrifices were conducted on PND 22 and after

completion of neurobehavioral evaluations on PND 74. Brain weight and measurements, neuropathology, and morphometric measurements were performed at both necropsies.

Several intercurrent deaths and clinical signs of toxicity were considered incidental to treatment. Body weight was slightly but significantly decreased at 800 mg/kg/day (males: 96-97%; females: 95-97% of control value) and 1000 mg/kg/day (males: 95-97%; females: 93-96% of control value). Cumulative body weight gain during the dosing period was also significantly decreased in males at 800 and 1000 mg/kg/day (79-95% and 73-94% of control value, respectively). Cumulative body weight gain during the dosing period was significantly decreased in females at 800 and 1000 mg/kg/day (86-93% and 76-90% of control value, respectively). Body weight and weight gain in the post-dosing period were comparable between treated and control animals. Food consumption was not affected by treatment.

The mean age of sexual maturation (preputial separation in males and vaginal opening in females) was not affected by treatment. No statistically significant changes between control and treated animals were observed in the behavioral assessment parameters, including FOB, motor activity, auditory startle reflex habituation, passive avoidance testing, and watermaze performance. No treatment-related effects were observed in mean brain weights (absolute and relative) and measurements of the cerebrum and cerebellum on PNDs 22 and 74. No treatment-related effects were observed on the neuropathology and morphometric parameters at either the PND 22 or 74 necropsy.

The systemic toxicity LOAEL in rats exposed percutaneously to p-Menthane-3,8-diol on PNDs 10 through 21 was 800 mg/kg/day based on decreased body weight and body weight gain. The systemic NOAEL was 400 mg/kg/day.

The neurotoxicity LOAEL in rats exposed percutaneously to p-Menthane-3,8-diol on PNDs 10 through 21 was not established. The neurotoxicity NOAEL was ≥ 1000 mg/kg/day.

This study is classified **Acceptable/Non-guideline**; it was intended to evaluate the potential for neurotoxic effects in offspring after direct percutaneous exposure to p-Menthane-3,8-diol during the preweaning period.

COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Technical grade para-menthane-3,8-diol

Description:	"Clearly and slightly viscous liquid, may solidify at room temperature"
Lot #/Batch #:	Lot # 061402Ad; Batch # 463D3
Purity:	99.63% a.i. (62.66% cis-p-Menthane-3,8-diol; 36.97% trans-p-Menthane-3,8-diol)
Compound Stability:	Stable for 12 months at room temperature (Appendix J)

2. **Vehicle and/or positive control:** The test article was applied neat; no vehicle was used. Control animals received reverse osmosis membrane processed deionized water. No positive control was used.

3. **Test animals (P):**

Species:	Rat
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®
Age at study initiation:	Postnatal day (PND) 10
Wt. at study initiation:	males: 14.6-26.1 g; females: 13.1-25.9 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	Housed in common nesting box during postpartum period; individually in stainless steel, wire-bottomed cages after weaning
Diet:	Certified Rodent Diet® 5002 (PMI Nutrition International, Inc.), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 74-82°F (target) **Humidity:** 30-70% (target) **Air changes:** 10/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	Approximately five days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: March 16, 2003; End: May 22, 2003

2. **Study schedule:** Crl:CD®(SD)IGS BR VAF/Plus® pups (80/sex/dose group) were administered the test material by percutaneous application from postnatal day (PND)10 through 21. (The day of birth was designated as PND 1.) One pup/sex/litter was assigned to the following evaluations: PND 22 brain weight and neurohistology; watermaze and passive avoidance: motor activity and acoustic startle reflex habituation; and brain weight and neuropathology at sacrifice on PND 74.

3. **Animal assignment:** Maternal animals with their litters were delivered to the test facility on PNDs 2 - 5. On PND 8, litters were reduced to four pups/sex using a table of random units. After standardization, litters were assigned to four dose groups (Groups I through IV), 20 litters/dose group (4 pups/sex/litter; 80 pups/sex/group) based on computer generated weight ordered randomization procedures. Mean body weight of each litter on PND 8 was used for randomization. One male and one female pup were assigned to the groups shown in Table 1.

TABLE 1. Study design[a]				
Experimental parameter	Dose (mg/kg/day)/Dosage volume (mL/kg)			
	0/1.0	400/0.4	800/0.8	1000/1.0
	No. of pups assigned			
Brain weight (PND 22) - Subset 1	10/sex	10/sex	10/sex	10/sex
Neurohistology (PND 22) - Subset 1	10/sex	10/sex	10/sex	10/sex
Watermaze (PNDs 61±2 and 68±2) - Subset 2	20/sex	20/sex	20/sex	20/sex
Passive avoidance (PNDs 24±1 and 31±1) - Subset 2	20/sex	20/sex	20/sex	20/sex
Motor activity (PNDs 14, 18, 22 and 61±2) - Subset 3	20/sex	20/sex	20/sex	20/sex
Acoustic startle habituation (PNDs 23 and 61±2) - Subset 3	20/sex	20/sex	20/sex	20/sex
FOB (PNDs 12, 22, 36, 46, and 61) - Subset 4	20/sex	20/sex	20/sex	20/sex
Brain weight (PND 74) - Subset 4	10/sex	10/sex	10/sex	10/sex
Neurohistology (PND 74) - Subset 4	10/sex	10/sex	10/sex	10/sex

[a] Data obtained from page 23 of MRID 46342801.

4. **Dose selection rationale:** The dose selection was based on a previous study but the study design and results were not provided.

5. **Dosage administration:** The test material was applied to the backs of the pups on PNDs 10 through 21. Dosing was based on the most recent body weight and was given at approximately the same time each day. Control animals received reverse osmosis membrane processed deionized water. Each application site was approximately 1.8 cm in diameter. Both the test material and control substance were warmed to 40°C before use. The application site was occluded a with Hilltop Chamber® to prevent oral ingestion and minimize the loss of the test material. During the exposure period, the pups were housed by litter in separate nesting boxes while their dams were placed in individual housing. After a six hour exposure, the site was washed with soapy water and dried. A small amount of menthol material was applied to the nose of the dam and the top of each pup's head before the pups were returned to their dams.

6. **Dosage preparation and analysis:** The testing facility received a quantity of lot# 061402Ad from the manufacturer with a Certificate of Analysis. Samples of the test material used for the study (Batch 463D3) were obtained by sub-dividing lot# 061402Ad without formulation or dilution. An analysis of this batch which was stored at room temperature was conducted after completion of the study.

Results: The study report (Appendix J, page 453) states that the post-analysis (after completion of the study) found 99.3% p-Menthane-3,8-diol as compared to 99.63% a.i. in the original Certificate of Analysis from the manufacturer.

The analytical data indicated that the stability of p-Menthane-3,8-diol was adequate during the course of the study.

C. OBSERVATIONS:

1. In-life observations:

a. **F_0 generation females:** Dams were observed for viability at least twice per day and were examined for clinical signs and general appearance weekly. Maternal behavior was recorded daily. Body weight and food consumption were measured on lactation days (LDs) 10, 12, 15, 18, 20 and 22; however, these data were not reported.

b. **F_1 generation animals:**

1) **Litter observations:** Pups were checked for viability at least twice daily during the preweaning and postweaning periods. Clinical observations were recorded daily before dosing. Post-dosing observations were recorded within one to two hours after rinsing the application site.

Before the first topical application and at 24-hour intervals each day thereafter during the dosing period, each skin site was examined for signs of irritation and scored using the Draize and National Research Council grading systems.

Body weight was recorded on the day after arrival at the testing facility, on PND 8, daily during the dosing period, weekly during the post-dosing period and prior to sacrifice. Food consumption was recorded weekly during the post-dosing period.

2) **Developmental landmarks:** Beginning on PND 39, male pups in Subsets 2 through 4 were examined for preputial separation. Beginning on PND 28, female pups in Subsets 2 through 4 were examined for vaginal patency. The age of onset was recorded but body weight at attainment was not recorded.

3) **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.

i) **Functional observational battery (FOB):** On PNDs 12, 22, 36, 46 and 61, twenty offspring/sex/group in Subset 4 were examined outside the home cage in an FOB assessment by a technician unaware of the rat's dosage group. The size of the arena for the FOB observations was not provided. Animals were evaluated for signs of autonomic dysfunction, abnormal posture, abnormal behavior patterns, and unusual appearance. Autonomic dysfunction evaluation consisted of an assessment of lacrimation, salivation, palpebral closure, prominence of the eye, piloerection, respiration, urination, and defecation. No other experimental details were provided.

ii) **Motor activity testing:** Motor activity was evaluated on PNDs 14 and 18 before dosing, and on PNDs 22 and 61 (± 2 days). One pup/sex from each litter in Subset 3 was tested. A passive infrared sensor mounted outside a stainless steel wire-bottomed cage (post-weaning) or Plexiglass bottom cage (pre-weaning) was used to monitor the movements of the pups. Each test session lasted one hour with the number of movements and time spent in movement being tabulated at each ten-minute interval.

iii) **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was evaluated on PNDs 23 and 61 (± 2 days) in one pup/sex from each litter in Subset 3 using a sound-attenuated chamber. Each rat was placed in a small cage above a platform containing a force transducer. A microcomputer sampled the output of the force transducer and controlled the test session. During the last minute of the five-minute adaptation period, ten "blank" trials were given to sample the baseline force without a stimulus. The rats were then tested with 30 msec, 120 dB bursts of noise at ten second intervals for 50 trials. An additional ten "blank" trials followed. The peak amplitude of each response was recorded and the average response in baseline trials was subtracted to calculate the response magnitude.

iv) **Learning and memory testing**

a) **Passive avoidance**: Passive avoidance testing assessing learning, short-term retention, long-term retention, and hyperactivity was conducted on PNDs 24±1 and 31±1 on one rat/sex from each litter in Subset 2. The testing apparatus consisted of a two-compartment chamber separated by a sliding door. One compartment was fitted with a bright light and Plexiglas floor. The other compartment was fitted with a grid floor to which a one second pulse of mild electric current (1mA) was delivered. For each trial, the rat was placed into the "bright" compartment, the sliding door was opened and the light was turned on. The rat was allowed to explore until it entered the "dark" compartment. The sliding door was then closed, the light turned off and the brief pulse of current delivered to the grid floor. The rat was then removed from the apparatus and put into a holding cage for 30 seconds until the next trial. Trials were repeated until the rat stayed in the "bright" compartment for 60 seconds on two consecutive trials (criterion for learning) or until 15 trials were completed. The latency (time) to enter the dark compartment or the maximum 60-second interval was recorded for each trial. Each rat was tested twice with the sessions being separated by one week. Dose groups were compared using the following measures: number of trials to the learning criterion in the first session (overall learning performance); time to enter the "dark" compartment from the "bright" compartment on trial 1 in the first session (activity level and exploratory tendency comparison); time to enter "dark" compartment from the "bright" compartment on trial 2 (short-term retention); number of trials to the learning criterion in the second session (long-term retention); and time to enter the "dark" compartment from the "bright" compartment in trial1 of the second session (long-term retention).

b) **Watermaze testing**: Watermaze testing for evaluation of overt coordination, swimming ability, learning and memory was conducted on PNDs 61±2 and 68±2 on one rat/sex from each litter in Subset 2. Each rat was tested in a watertight 16-gauge stainless steel modified M-maze filled with water approximately 9 inches deep with a temperature of 21°C ± 1°C. For each test, the rat was placed at the base of the M-maze stem farthest from the two arms and required to swim to one of the two goals of the M-maze in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm for trial 1 was the incorrect goal for the remaining trials. Rats that didn't make the correct choice within 60 seconds in any trial were guided to the correct goal. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials per session was 15. Time to choose the correct goal or the maximum 60-second interval and the number of errors (incorrect turns in the maze) were recorded for each trial. Each rat was tested twice with a one-week interval between sessions. Dose groups were compared using the following measures: number of trials to criterion on the first day of testing (overall learning performance); average number of errors for each trial on the first day of testing (overall learning performance); time to reach the correct goal on trial 2 of the first day of testing (short-term retention); number of trials to criterion on the second day of testing (long-term retention); average number of errors on each trial on the second day of testing (long-term retention); and time to reach the correct goal on trial 1 of the second day of testing (long-term retention).

2. **Postmortem observations**:

a. **F_0 generation females:** On LD 22, dams with litters assigned to study were sacrificed and discarded without post-mortem examination.

b. **F_1 generation animals**: Pups sacrificed on or before PND 14 and those selected for neurohistopathological evaluation (Subsets 1 and 4) were euthanized by intraperitoneal injection of sodium pentobarbital. Otherwise, rats were euthanized with carbon dioxide asphyxiation. Pups not selected for continued study on PND 8 were sacrificed and discarded without examination. Pups and rats found dead or sacrificed in a moribund condition were examined for the cause of death or the moribund condition and gross lesions. F_1 generation rats were sacrificed either on PND 22 (Subset 1) or after completion of post-weaning behavioral evaluations. Rats in Subsets 1 and 4 not selected for neurohistological procedures were examined for gross lesions. The liver, kidneys (paired), thymus and spleen in the control and high dose groups were weighed and retained in fixative for possible future examination.

On PNDs 22 and 74, ten rats/sex/group were perfusion-fixed with 10% neutral buffered formalin (NBF) and necropsied at the testing facility. The brain (PNDs 22 and 74), spinal cord, and hindleg peripheral nerves (PND 74) were exposed but left *in situ*. All tissues were then placed back into NBF for further fixation and shipment to Pathology Associates International (PAI) for further dissection and histologic procedures. At PAI, the brain was removed and weighed. (There was a delay in shipment of the tissues from the low and intermediate dose groups necropsied on PND 70 so that the brains from these groups were in the fixative nine weeks longer than the brains from the control and high dose groups.) Linear measurements of the brain, taken using a Vernier caliper, were as follows: 1) length of the cerebrum from the anterior to posterior pole, exclusive of the olfactory bulbs; and 2) linear measurement of the cerebellum extending from the anterior edge of the cerebellar cortex to the posterior pole. The measurements were done by an individual blinded to the dose group assignments. After the brain measurements were completed, the brain was sliced, processed, and embedded in paraffin. Nine multiple coronal slices were cut; the first six cuts were made starting from the ventral aspect of the brain and the seventh through ninth made from the dorsal surface.

8

After removal of the brain from the rats in the control and high dose groups destined for histopathologic evaluation, the Gasserian ganglia and associated trigeminal nerve tissues were removed from the PND 74 rats. The spinal cord and ventral nerve roots were removed from the vertebral columns at PAI. Two cross-sections of the spinal cord were taken from each of the cervical and lumbar swellings, as well as from the mid-thoracic cord. One horizontal and one paramedian sagittal section were also taken of the cervical spinal cord. The sciatic nerve and its branches were removed from the one of the hind legs of control and high dose rats sacrificed on PND 74. Cross and longitudinal sections of the sciatic nerve and tibial nerve and longitudinal sections of the common peroneal nerve and sural nerves were processed. The slices of brain, spinal cord, Gasserian ganglia, nerve roots and dorsal root ganglia were embedded in paraffin (listed below). Segments of the peripheral nerves were embedded in glycol methacrylate (listed below). Brain sections from PND 22 rats were stained with hematoxylin and eosin (H & E) and luxol fast blue/cresyl violet (LFB/CV). Paraffin-embedded tissues from PND 74 rats were stained with H & E, LFB/CV and Bielschowsky's technique (silver stain for axons and neuronal cytoarchitecture). Tissues from the glycol methacrylate blocks were stained with H & E, toluidine blue and Bielschowsky's technique. Microscopic slides were prepared at PAI and then sent to Consultants in Veterinary Pathology, Inc. (CVP) for microscopic examination.

Blocks for paraffin-embedded tissues:
1) Coronal slice through the cerebrum at the level of the optic chiasm.
2) Coronal slice through the cerebrum at the level of the infundibulum.
3) Coronal slice through the cerebrum at the level of the mammary bodies.
4) Coronal slice through the middle of the cerebellum.
5) Sections including the olfactory bulbs, two coronal slices through the anterior pole, one coronal slice through the cerebrum at the level of the midbrain, one through the posterior portion of the cerebellum and one through the medulla oblongata.
6) Longitudinal sections of the Gasserian ganglia and associated trigeminal nerves.
7) Longitudinal sections of the dorsal root ganglia and spinal nerve roots.
8) Cross and longitudinal sections of the spinal cord.

Blocks for glycol metharylate-embedded tissues:
9) Longitudinal section of the sciatic nerve plus cross sections of the sciatic and tibial nerves.
10) Longitudinal sections of the common peroneal, tibial and sural nerves.

Only blocks # 1-5 were prepared for PND 22 rats.

Thirteen brain morphometric measurements were performed on each brain processed for microscopic evaluation. Two of these were gross linear measurements done at PAI. The other eleven represented six regions (listed below), five of which were measured bilaterally. For measurements 1 through 5 in the list below, measurements were taken bilaterally and recorded separately; mean values were used for statistical analyses.

1) Thickness of the frontal cortex.
2) Thickness of the parietal cortex.

3) Diagonal width of the caudate putamen and underlying globus pallidus; this measurement was also performed on the coronal section at the level of the optic chiasm.
4) Thickness of the corpus callosum just lateral to its mid point within the section taken at the level of the optic chiasm.
5) Thickness of the hippocampal gyrus; measurement was taken within section passing through infundibulum.
6) Maximum height of the cerebellum at the level of the deep cerebellar nuclei.

Histopathology sections were examined from each rat but because of the large number of neuroanatomic regions in the nervous system, only selected regions (32 for PND 22 and 42 for PND 74 rats) were entered into the computer data base. A summation of the data was presented in ten sections, proceeding from anterior to posterior and using Bregma measurements corresponding to the brain level in a standard atlas of adult rat neuroanatomy.[1] All microscopic findings were graded on a scale of 1 to 5 (minimal, mild, moderate, marked or severe). All lesions were also qualified by distribution (focal, multifocal or diffuse).

D. DATA ANALYSIS:

1. **Statistical analyses**: The individual rat was the unit of measure for analyses. Variables with interval or ratio scales of measurement, including body weight, food consumption, latency, and errors per trial scores in behavioral tests, and percent mortality per litter, were first analyzed using Bartlett's Test of Homogeneity of Variances to estimate the probability that the dose groups have different variances. A non-significant result ($p>0.001$) indicated that an assumption of homogeneity of variance was not inappropriate and the data were compared using the Analysis of Variance Test (ANOVA). If that test was signficant ($p<0.05$), treated and control groups were compared using the Dunnett's Test. If the Bartlett's Test was significant ($p\leq0.001$), the ANOVA was not appropriate and nonparametric tests were used. When 75% or fewer of the scores in all groups were tied, the Kruskal-Wallis Test was used to analyze the data and in the event of a significant result ($p<0.05$), Dunn's Test was used to compare treated and control groups. When more than 75% of the scores in any dosage group were tied, Fisher's Exact Test was used to compare the proportion of ties in the dosage groups.

Data from motor activity and acoustic startle reflex habituation tests, with measurements recorded at intervals (blocks) were analyzed using ANOVA with repeated measures. A significant result ($p<0.05$) in that test can appear as an effect of dose or as an interaction between dose and block. If the dose effect was significant, the totals for the control and treated groups were compared using Dunnett's Test. If the dose x block interaction was significant, an ANOVA was used to evaluate the data at each measurement period and a significant result ($p<0.05$) was followed by a comparison of the groups using Dunnett's Test.

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[1] Paxinos, G and Watkins, C. (1997): The Rat Brain in Stereotaxic Coordinates, Compact Third Edition, Academic Press, N.Y.

Variables with graded or count scores, such as the number of trials to a criterion in a behavioral test or the day a developmental landmark appeared, were analyzed using procedures described above for nonparametric tests.

Clinical observation incidence data, as well as other proportion data, were analyzed as contingency tables using the Variance Test for Homogeneity of the Binomial Distribution.

2. **Positive and historical control data:** Testing facility positive and historical control data have been submitted to EPA in electronic CD format. These data have been reviewed with the conclusion that Argus Research Laboratories, Inc. has demonstrated proficiency in developmental neurotoxicity testing.

II. RESULTS:

A. Mortality and clinical and functional observations: One, two and three males and one, two and one females in the 400, 800 and 1000 mg/kg/day groups, respectively, were found dead or sacrificed in moribund condition during the study. The conditions of the deaths/sacrifice are described below.

400 mg/kg/day

A male pup was found dead approximately 2 hours after the eighth dose on PND 17. There were no clinical signs prior to death, all skin observations were normal and the pup gained weight. The cause of death could not be determined. A female pup was sacrificed in moribund condition before the second dose on PND 11. Clinical signs were related to an injury of the right hindlimb that occurred on the first day of dosing.

800 mg/kg/day

A male pup was found dead approximately 4.5 hours after the first dose on PND 10. There were no clinical signs and the cause of death could not be determined. Another male rat was sacrificed in moribund condition on PND 71. Adverse clinical signs were related to trauma when the animal attempted to free its entangled teeth from the caging. A female pup was found dead on PND 21 prior to dosing. The necropsy revealed thickened bladder walls and bilateral dilation of the renal pelvis. The cause of death was presumed to be due to the urinary tract lesions. A female rat was found dead on PND 29. The only clinical sign was sparse hair coat. The cause of death could not be determined.

1000 mg/kg/day

One male pup was found dead on PND 22 approximately 28.5 hours after the last dose. The death was considered accidental in that the rat was found with its head caught in the opening of the cage. Another male rat was sacrificed in moribund condition on PND 24 after it was found outside its cage. Adverse clinical signs included lost righting reflex, decreased motor activity, cold to touch, dehydration and gasping. The condition was considered to be the result of the lack of food and water when the animal was outside its cage overnight. A third male rat was found dead on PND 30. There were no clinical observations prior to death, skin observations were normal and the rat had gained weight. The cause of death could not be determined. A female rat was found dead on PND 45. Prior to death, the animal was cold to the touch, unconscious and emaciated. The cause of death was attributed to weight loss resulting from a jammed feed jar screen.

Clinical signs included a non-significant increased incidence of dehydration in male rats and of cold to touch in females, both at 1000 mg/kg/day. Most of these signs occurred in the rats found dead or sacrificed in moribund condition described above. Statistically significant increased incidences of the following signs were observed: sparse hair coat on the head and flaking (grade 1) in males and females at 800 mg/kg/day; red perioral substance in males at 800 mg/kg/day; ungroomed coat in males and females at 400 mg/kg/day. None of these clinical signs or skin observations is considered treatment-related as there was no dose response.

B. **Body weight, body weight gain and food consumption**: Selected group mean body weight, body weight gain and food consumption data are summarized in Table 2. Body weight was slightly but significantly decreased at 800 mg/kg/day (males: 96-97%; females: 95-97% of control value) and 1000 mg/kg/day (males: 95-97%; females: 93-96% of control value). Cumulative body weight gain during the dosing period (PNDs 10-22) was significantly decreased in males at 800 and 1000 mg/kg/day (79-95% and 73-94% of control value, respectively). Cumulative body weight gain during the dosing period was significantly decreased in females at 800 and 1000 mg/kg/day (86-93% and 76-90% of control value, respectively). Body weight and weight gain in the post-dosing period were comparable between treated and control animals. Food consumption, measured during the post-dosing period, was not affected by treatment.

TABLE 2. Selected mean (±SD) pup body weight, body weight gain and food consumption[a]								
PND	Dose (mg/kg/day)							
	0	400	800	1000	0	400	800	1000
	Males				Females			
Body weight (g)								
10	21.3±1.6	21.2±1.5	21.2±2.2	21.1±2.0	20.5±1.7	20.2±1.8	20.0±2.2	20.0±1.6
12	23.8±1.8	23.5±1.8	23.2±2.4	23.2±2.0	23.0±1.9	22.7±2.1	22.2*±2.5 (97)	22.1**±1.7 (96)
20	41.0±4.3	40.3±4.6	39.4*±4.6 (96)	38.9**±4.7 (95)	40.3±3.8	39.6±4.5	38.3**±4.8 (95)	37.3**±4.8 (93)
22	49.2±4.9	48.7±5.0	47.6±5.5	47.2±5.2 (96)	48.2±4.4	47.6±4.9	45.7**±5.8 (95)	44.8**±5.4 (93)
74	432.9±34.3	442.8±47.6	439.2±38.1	449.4±49.9	267.1±32.3	260.4±36.8	262.5±38.6	258.4±32.4
Body Weight Gain (g)								
11-12	1.4±0.5	1.3±0.5	1.1**±0.6 (79)	1.1**±0.8 (79)	1.4±0.4	1.4±0.5	1.2*±0.6 (86)	1.2**±0.6 (86)
16-17	2.2±1.0	1.9*±0.9 (86)	2.0±0.8 (91)	1.8*±0.8 (82)	2.2±0.8	2.1±0.7	1.9*±1.0 (86)	1.9**±0.8 (86)
10-22	27.8±4.8	27.4±4.5	26.4±4.6 (95)	26.0±5.0 (94)	27.7±4.2	27.4±4.2	25.7**±5.1 (93)	24.8**±4.9 (90)
10-74	411.6±34.0	421.7±47.1	417.9±37.7	428.3±49.4	246.7±32.2	240.3±36.2	242.6±38.6	238.5±32.3
Food Consumption (g/day)								
23-30	14.1±1.6	14.6±1.6	14.7±1.4	14.4±1.5	13.8±1.4	13.7±1.5	13.7±1.7	13.2±1.4
23-74	26.0±2.2	26.7±2.5	26.6±2.0	26.6±2.4	20.5±2.0	20.5±2.3	20.5±2.5	20.2±2.1

[a] Data obtained from pages 79-90, MRID 46342801.
PND = post-natal day
Number for body weight and body weight gain = 79-80 for PNDs 10-22, 57-60 for PNDs 23-74.
Number for food consumption = 50-60
* Statistically significantly different from control, $p < 0.05$.
** Statistically significantly different from control, $p < 0.01$.
Number in parentheses is % of control value, calculated by reviewer.

C. Developmental landmarks:

Sexual maturation: The mean age of preputial separation in males was 46.3, 46.1, 45.7 and 45.8 days for the control, 400, 800 and 1000 mg/kg/day groups, respectively. The mean age of vaginal opening in females was 32.0, 31.9, 32.0 and 32.2 days for the respective groups. Body weight at attainment was not reported. The data are presented in Table 3.

TABLE 3. Mean (±SD) age (days) at sexual maturation[a]				
Parameter	Dose (mg/kg/day)			
	0	400	800	1000
N (M/F)	60/60	59/59	59/58	57/60
Preputial separation age	46.3±2.3	46.1±1.7	45.7±2.1	45.8±1.8
Vaginal opening age	32.0±1.5	31.9±2.0	32.0±2.0	32.2±1.9

[a] Data obtained from page 91, MRID 46342801.

D. Behavioral assessment:

1. **Functional observational battery**: There were no treatment-related FOB findings on PNDs 12, 22, 36. 46 and 61.

2. **Motor/locomotor activity:** Total number or movements and total time spent in movement for Subset 3 animals are presented in Table 4. No treatment-related effects were observed in males or females on PNDs 14, 18, 22 or 60. Habituation was generally observed on PNDs 14, 18, and 22, but not on PND 60.

TABLE 4. Mean motor activity data (mean ±SD) [a]

Test Day	Dose (mg/kg/day)			
	0	400	800	1000
Males				
PND 14				
Total # movements	372.0±154.8	359.6±173.7	387.8±168.8	392.6±186.2
Total # time (secs) moving	467.0±223.3	460.0±301.8	503.4±291.5	491.4±264.0
PND 18				
Total # movements	417.0±193.5	580.8±209.5	532.2±207.4	531.4±201.6
Total # time (secs) moving	670.4±386.4	928.2±387.9	858.2±429.9	910.9±500.4
PND 22				
Total # movements	430.2±165.6	473.0±163.3	467.1±122.2	451.5±175.0
Total # time (secs) moving	705.7±323.4	790.4±347.1	781.5±172.1	799.8±349.0
PND 60				
Total # movements	793.7±80.5	804.6±87.2	799.6±51.4	822.5±72.6
Total # time (secs) moving	1844.5±277.8	1872.2±271.3	1918.7±238.5	1920.7±253.0
Females				
PND 14				
Total # movements	443.4±152.0	451.0±140.0	417.3±168.8	409.4±167.0
Total # time (secs) moving	609.8±276.7	647.1±299.4	533.7±280.3	566.7±290.4
PND 18				
Total # movements	532.9±220.1	560.4±180.0	530.4±191.4	489.5±232.5
Total # time (secs) moving	902.2±464.1	928.8±382.0	878.8±403.4	809.7±459.3
PND 22				
Total # movements	429.0±163.5	435.3±158.6	468.4±134.0	473.9±167.1
Total # time (secs) moving	720.6±298.2	718.5±291.0	857.4±363.9	831.0±337.5
PND 60				
Total # movements	785.8±95.6	808.3±104.6	771.4±115.3	769.5±99.7
Total # time (secs) moving	1843.6±386.3	1812.3±288.4	1858.0±420.1	1840.4±316.5

[a] Data obtained from pages 288-295, MRID 46342801
N = 19-20

3. **Auditory startle reflex habituation** : The average response magnitude data are presented in Table 5. No statistically significant differences were observed between control and treated Subset 3 animals on PNDs 23 and 61. Habituation was evident on both testing days.

TABLE 5. Auditory startle data (g ±SD) [a]

Response Magnitude[b]	Dose (mg/kg/day)			
	0	400	800	1000
Males				
PND 23	15.4±7.3	15.7±10.6	18.3±9.0	18.3±10.9
PND 61	53.5±37.8	61.4±42.7	50.8±25.8	51.5±20.9
Females				
PND 23	13.9±4.6	13.9±9.5	15.0±7.8	16.8±12.4
PND 61	28.2±24.2	24.4±13.3	37.3±31.7	33.8±19.1

[a]Data obtained from 296-297, MRID 46342801.
[b] Response Magnitude = Peak Response - Baseline Response
N= 19-20

4. Learning and memory testing

a. **Passive avoidance testing:** The passive avoidance performance data in Subset 2 animals on PNDs 24±1 and 31±1 are summarized in Table 6. No statistically significant differences occurred in the number of trials to criterion, trial latencies or number of rats that failed to learn.

TABLE 6. Passive avoidance performance data (mean ±SD) [a]

Parameter	Dose (mg/kg/day)			
	0	400	800	1000
Males				
Session 1 (PND 24±1)[b]				
Trials to criterion	4.2±1.2	5.2±2.5	5.4±4.1	3.9±0.7
Latency trial 1 (secs)	9.4±4.8	8.9±5.8	10.0±4.2	10.4±5.4
Latency trial 2 (secs)	38.3±20.3	31.3±20.9	39.5±19.8	38.8±21.8
No. failed to learn (%)[c]	0 (0.0)	1 (5.3)	2 (10.5)	0 (0.0)
Session 2 (PND 31±1)[b]				
Trials to criterion	2.8±0.9	4.0±3.4	3.4±1.2	3.2±0.8
Latency trial (secs)	37.1±23.5	37.3±23.4	21.9±19.6	36.7±23.3
Females				
Session 1 (PND 24±1)[b]				
Trials to criterion	4.6±1.0	4.4±1.7	4.7±2.4	4.6±1.5
Latency trial 1 (secs)	9.9±4.8	10.6±5.0	7.1±3.7	9.6±7.0
Latency trial 2 (secs)	34.4±17.6	38.8±18.4	33.4±23.4	36.3±22.2
No. failed to learn (%)[c]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Session 2 (PND 31±1)[b]				
Trials to criterion	3.1±0.6	3.0±0.9	3.5±2.1	3.5±1.8
Latency trial (secs)	27.0±22.0	35.0±20.3	24.2±19.5	28.4±22.3

[a]Data obtained from 267-268, MRID 46342801
[b] Session 1 = learning phase; Session 2 = retention phase
[c] Number of rats that did not meet criterion in Session 1 (learning phase); Session 2 (retention phase) values for these rats were excluded from group averages and statistical analyses.
N= 17-20

b. **Watermaze performance:** The watermaze performance data for Subset 2 animals on PNDs 61±1 and 68±1 are summarized in Table 7. No statistically significant differences occurred in the number of trials to criterion, number of errors per trial, trial latencies, or numbers of animals that failed to learn.

TABLE 7. Watermaze performance data (mean ±SD) [a]

Parameter	Dose (mg/kg/day) 0	400	800	1000
Males				
Session 1 (PND 61±1)[b]				
Trials to criterion	9.1±3.1	9.5±2.8	9.5±2.7	8.6±2.2
No. errors per trial	0.5±0.3	0.5±0.2	0.5±0.3	0.5±0.3
Latency trial (secs)	18.4±9.2	15.2±7.4	14.5±8.9	17.3±15.7
No. failed to learn (%)[c]	1 (5.0)	1 (5.3)	1 (5.3)	0 (0.0)
Session 2 (PND 68±1)[b]				
Trials to criterion	6.3±2.7	5.5±0.7	5.9±2.0	6.2±2.6
No. errors per trial	0.1±0.2	0.1±0.1	0.1±0.1	0.1±0.2
Latency trial (secs)	9.6±5.1	10.7±6.5	8.2±4.3	10.3±8.7
Females				
Session 1 (PND 61±1)[b]				
Trials to criterion	8.1±2.1	9.0±2.2	7.7±1.8	8.6±2.1
No. errors per trial	0.4±0.2	0.4±0.1	0.4±0.2	0.4±0.2
Latency trial (secs)	12.0±6.4	11.2±5.2	12.3±7.2	13.6±11.0
No. failed to learn (%)[c]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Session 2 (PND 68±1)[b]				
Trials to criterion	7.0±2.7	6.8±2.4	6.1±2.0	5.7±1.6
No. errors per trial	0.1±0.1	0.1±0.2	0.1±0.1	0.1±0.1
Latency trial (secs)	7.8±4.0	8.8±6.8	8.8±6.5	8.0±5.8

[a] Data obtained from 269-270, MRID 46342801
[b] Session 1= learning phase; Session 2 = retention phase
[c] Number of rats that did not meet criterion in Session 1 (learning phase); Session 2 (retention phase) values for these rats were excluded from group averages and statistical analyses.
N= 18-20

5. Postmortem results:

a. **Brain weight, width and length:** Mean terminal body weight, brain weights (absolute and relative), and measurements of the cerebrum and cerebellum on PNDs 22 and 74 are presented in Table 8. The brain weight presented in the table is fresh weight measured at the testing facility. Fixed brain weight was measured at Consultants in Veterinary Pathology. No treatment-related differences were found for either fresh or fixed brain weights between the groups. No treatment-related effects were noted in any of the brain measurements. Significant decreases in fresh brain weights in male rats at 400 and 800 mg/kg/day on PND 22 and a decrease in cerebrum length in males at 800 mg/kg/day on PND 74 were not considered treatment-related since there were no effects at 1000 mg/kg/day.

TABLE 8. Mean (±SD) brain weight and gross measurement data [a]				
Parameter	Dose (mg/kg/day)			
	0	400	800	1000
Males				
Day 22				
Terminal body weight (g)	49.5±3.5	50.8±5.1	45.9±5.1	47.6±6.8
Brain weight (g)	1.62±0.07	1.54*±0.06	1.49**±0.06	1.59±0.08
Brain-to-body weight ratio (%)	3.28±0.21	3.05±0.25	3.28±0.30	3.39±0.42
AP of cerebrum (mm)	13.74±0.31	13.91±0.34	13.82±0.47	13.86±0.31
AP of cerebellum (mm)	5.41±0.47	5.58±0.51	5.55±0.19	5.31±0.33
Day 74				
Terminal body weight (g)	447.4±25.8	442.6±50.4	448.2±43.6	446.7±47.6
Brain weight (g)	2.12±0.08	2.11±0.10	2.18±0.13	2.13±0.11
Brain-to-body weight ratio (%)	0.48±0.03	0.49±0.06	0.49±0.03	0.48±0.05
AP of cerebrum (mm)	16.07±0.56	15.84±0.48	15.57*±0.43	15.91±0.43
AP of cerebellum (mm)	7.09±0.33	6.95±0.21	7.03±0.39	7.05±0.51
Females				
Day 22				
Terminal body weight (g)	48.6±3.6	47.8±4.4	47.6±5.9	46.7±3.4
Brain weight (g)	1.55±0.10	1.53±0.08	1.53±0.10	1.53±0.08
Brain-to-body weight ratio (%)	3.20±0.16	3.22±0.22	3.26±0.46	3.28±0.22
AP of cerebrum (mm)	13.63±0.61	13.73±0.41	13.77±0.42	13.83±0.43
AP of cerebellum (mm)	5.33±0.29	5.30±0.38	5.40±0.34	5.31±0.17
Day 74				
Terminal body weight (g)	261.8±21.1	262.2±28.6	279.5±45.3	247.0±27.7
Brain weight (g)	2.04±0.14	2.02±0.09	2.04±0.08	1.97±0.09
Brain-to-body weight ratio (%)	0.78±0.06	0.78±0.07	0.75±0.12	0.80±0.07
AP of cerebrum (mm)	15.45±0.60	15.43±0.51	15.29±0.53	15.49±0.38
AP of cerebellum (mm)	6.79±0.47	7.06±0.28	6.92±0.34	6.83±0.39

[a] Data obtained from pages 248-252, 379-383 and 549-552, MRID 46342801
AP = anterior to posterior
N=10-11
** Statistically significantly different from control, p≤0.01.

b. **Organ weights**: Selected organ weight values are in Table 9. At the PND 22 necropsy, female rats treated with 1000 mg/kg/day had increased absolute (N.S.) and relative ($p<0.01$) liver weights (111% and 115% of control value, respectively). At the PND 74 necropsy, females treated with 1000 mg/kg/day had decreased absolute ($p<0.05$) and relative (N.S.) spleen weights (84 and 89% of control value). On PND 74, the absolute thymus weight was significantly decreased in females treated with 400 and 1000 mg/kg/day; relative weight was significantly decreased in all treated groups. These changes are not considered treatment-related since there was no dose-response relationship.

TABLE 9. Mean (±SD) weights of selected organs [a]				
Parameter	Dose (mg/kg/day)			
	0	400	800	1000
Males				
Day 22				
Terminal body weight (g)	49.5±3.5	50.8±5.1	45.9±5.1	47.6±6.8
Liver Absolute weight (g) Relative weight (%)	2.86±0.28 5.79±0.53	2.83±0.46 5.56±0.50	2.52±0.35 5.50±0.46	2.77±0.42 5.83±0.50
Day 74				
Terminal body weight (g)	447.4±25.8	442.6±50.4	448.2±43.6	446.7±47.6
Spleen Absolute weight (g) Relative weight (%)	0.96±0.11 0.21±0.02	0.89±0.17 0.20±0.04	0.97±0.09 0.22±0.02	0.90±0.16 0.20±0.03
Thymus Absolute weight (g) Relative weight (%)	0.97±0.12 0.22±0.03	0.88±0.14 0.20±0.02	0.89±0.20 0.20±0.04	0.78±0.21 0.18±0.05
Females				
Day 22				
Terminal body weight (g)	48.6±3.6	47.8±4.4	47.6±5.9	46.7±3.4
Liver Absolute weight (g) Relative weight (%)	2.52±0.34 5.20±0.66	2.71±0.43 5.65±0.60	2.66±0.40 5.60±0.61	2.79±0.32 (111) 6.00**±0.43 (115)
Day 74				
Terminal body weight (g)	261.8±21.1	262.2±28.6	279.5±45.3	247.0±27.7
Spleen Absolute weight (g) Relative weight (%)	0.69±0.13 0.27±0.05	0.64±0.08 0.24±0.04	0.77±0.14 0.28±0.04	0.58*±0.07 (84) 0.24±0.03 (89)
Thymus Absolute weight (g) Relative weight (%)	0.75±0.14 0.29±0.04	0.53**±0.06 0.21**±0.03 (72)	0.68±0.12 0.25*±0.03 (86)	0.62*±0.08 (83) 0.25*±0.03 (86)

[a] Data obtained from pages 248-252 and 379-383, MRID 46342801
N=10-11
* Statistically significantly different from control, p ≤ 0.05.
** Statistically significantly different from control, p ≤ 0.01.

c. **Macroscopic and microscopic examination of non-neural tissues:** Gross lesions were collected and placed in fixative by the testing facility and then submitted to Research Pathology Services, Inc., for tissue processing, microscopic preparation, and histopathologic evaluation. The most common gross observation was unilateral or bilateral pelvic dilatation of the kidney, which was observed in both control and treated groups with no dose response. All of the gross lesions in the kidney were confirmed microscopically.

d. **Neuropathology:**

1) **Microscopic examination:** No microscopic alterations were observed in rats necropsied on PND 22. One control rat at the PND 74 necropsy had a focus of malacia within the superior anterior frontal cortex which was considered a focus of traumatic injury or ischemia. At the PND 74 necropsy, three male control animals had degeneration of the

18

tibial nerve and one female treated with 1000 mg/kg/day had degeneration of one spinal nerve root. Foci of neuron cytoplasmic vacuolation within the Gasserian ganglia were observed in one control female, one male at 1000 mg/kg/day and three females at 1000 mg/kg/day at the PND 74 necropsy. One female at 1000 mg/kg/day had vacuolated neurons in the dorsal root ganglia.

2) **Brain morphometry:** Morphometric evaluation data are presented in Table 10. Hippocampal thickness was decreased in male rats at 1000 mg/kg/day at the PND 22 necropsy. This change is not considered treatment-related since there was no effect in treated females at the PND 22 necropsy or in either sex at the PND 74 necropsy.

TABLE 10. Mean (μ±SD) brain morphometric data [a]

Parameter	Dose (mg/kg/day)			
	Males		Females	
	0	1000	0	1000
Day 22				
Frontal cortex	1899±44.83	1887±71.34	1902±58.65	1857±45.72
Parietal cortex	1908±66.63	1869±49.09	1896±46.48	1890±50.99
Striatum (caudate-putamen)	2990±137.73	2904±68.82	2962±71.73	2904±85.42
Corpus callosum	188±35.80	176±28.87	163±12.90	154±13.62
Hippocampus	1275±49.50	1209**±51.09 (95)	1272±40.50	1272±35.21
Cerebellum	4950±230.22	4920±217.26	4914±86.95	4872±132.06
Day 74				
Frontal cortex	1866±70.43	1866±46.48	1830±60.00	1782±55.14
Parietal cortex	1908±96.12	1887±65.50	1836±61.32	1809±49.09
Striatum (caudate-putamen)	3130±67.12	3120±106.13	3014±125.58	2962±110.97
Corpus callosum	262±25.8	252±17.89	236±31.01	242±31.39
Hippocampus	1413±53.76	1410±37.42	1380±70.71	1326±54.41
Cerebellum	5226±261.45	5322±183.41	4944±287.33	4860±189.74

[a] Data obtained from pages 553-556, MRID 46342801.
N = 10
** Significantly different from control, p<0.01

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that the No Observed Adverse Effect Level (NOAEL) for toxicity was 400 mg/kg/day based on statistically significant reductions in body weight gain in males and females treated at 800 and 1000 mg/kg/day. The NOAEL for developmental neurotoxicity was greater than 1000 mg/kg/day, the highest dose tested.

B. **REVIEWER COMMENTS:** One, two and three males and one, two and one females in the 400, 800 and 1000 mg/kg/day groups were found dead or sacrificed in moribund condition during the study. The cause of death, as determined by necropsy or pre-mortem accidental events, was not treatment-related in one female at 400 mg/kg/day, one male and one female at 800 mg/kg/day and two males and one female at 1000 mg/kg/day. The remaining deaths (1 male at 400 mg/kg/day, 1 male and 1 female at 800 mg/kg/day and 1 male at 1000 mg/kg/day) are not considered treatment-related because there was no dose response.

Body weight and body weight gain were significantly decreased in males and females at 800 and 1000 mg/kg/day. The decrease in weight gain appeared to be biphasic with an initial effect after the beginning of treatment on PND 10, then another effect after PND 16 when the pups would have been transitioning to food. Although the absolute body weight of the treated pups did not differ from that of the controls by more than 10% during lactation, the decreases in both body weight and body weight gain are considered biologically significant in growing pups. No effects on body weight or body weight gain were noted after the dosing interval and the pups were weaned. Food consumption, which was measured during the post-dosing period, was not affected by treatment.

The mean age of sexual maturation (preputial separation in males and vaginal opening in females) was not affected by treatment. No statistically significant changes between control and treated animals were observed in the behavioral assessment parameters, including FOB, motor activity, auditory startle reflex habituation, passive avoidance testing, and watermaze performance.

No treatment-related effects were observed in mean brain weights (absolute and relative) or measurements of the cerebrum and cerebellum on PNDs 22 and 74. Significant decreases in fresh brain weight in male rats treated with 400 and 800 mg/kg/day on PND 22 and a decrease in cerebrum length in males treated with 800 mg/kg/day on PND 74 were not considered treatment-related since there were no effects at 1000 mg/kg/day. At the PND 22 necropsy, female rats at 1000 mg/kg/day had increased relative ($p<0.01$) liver weight which probably reflect the slightly lower terminal body weight for this group. At the PND 74 necropsy, females at 1000 mg/kg/day had decreased absolute ($p<0.05$) and relative (N.S.) spleen weights. Also on PND 74, the absolute thymus weight was significantly decreased in females at 400 and 1000 mg/kg/day; relative weight was significantly decreased in all treated groups. These organ weight changes are not considered treatment-related since there was no dose-response relationship. No treatment-related effects on the incidence of gross lesions were observed. The most common gross observation was unilateral or bilateral pelvic dilatation of the kidney, which was observed in both control and treated groups with no dose response. All of the gross lesions in the kidney were confirmed microscopically.

No treatment-related effects were observed on the neuropathology parameters at either the PND 22 or 74 necropsy. The findings were either sporadic or there was no dose response relationship. One control rat at the PND 74 necropsy had a focus of malacia within the superior anterior frontal cortex which was considered a focus of traumatic injury or ischemia. Findings at the PND 74 necropsy, including degeneration of the tibial nerve or spinal nerve root and neuron cytoplasmic vacuolation, are considered common alterations and not treatment-related.

The systemic toxicity LOAEL in rats exposed percutaneously to p-Menthane-3,8-diol on PNDs 10 through 21 was 800 mg/kg/day based on decreased body weight and body weight gain. The systemic NOAEL was 400 mg/kg/day.

The neurotoxicity LOAEL in rats exposed percutaneously to p-Menthane-3,8-diol on PNDs 10 through 21 was not established. The neurotoxicity NOAEL was ≥ 1000 mg/kg/day.

C. **STUDY DEFICIENCIES:**. None

DATA FOR ENTRY INTO ISIS

Postnatal Developmental Neurotoxicity Study - rats (non-guideline)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range (mg/kg/day)	Doses tested (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Target organ(s)	Comments
011550	46342801	postnatal developmental neurotoxicity	rat	PND 10-21	dermal	dermal	0-1000	0, 400, 800, 1000	400	800	body weight, body weight gain	systemic toxicity
011550	46342801	postnatal developmental neurotoxicity	rat	PND 10-21	dermal	dermal	0-1000	0, 400, 800, 1000	>1000	not established		neurotoxicity